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Refractive Index of Fly Rhabdomeres

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Summary. The refractive index reported by Seitz (1968) for the rhabdomeres of flies (1.349) has been corrected for waveguide effects. The presented correction method has yielded $n_1 = 1.365 \pm 0.006$.

It is argued that an acceptable estimate for the refractive index of the inhomogeneous surroundings of fly rhabdomeres is $n_2 = 1.339 \pm 0.002$.

A. Introduction

A fly rhabdomere has a higher refractive index than its surrounding medium as is generally the case for photoreceptors. Therefore these structures act as optical waveguides. A characteristic feature of (dielectric) waveguides is that the light wave is conducted in modes, i.e. light patterns extending across the boundary of the fibre. The fraction of the wave propagated within the borders depends on wavelength, fibre radius and refractive indices of both the medium within the fibre and that surrounding it.

The phenomenon of the boundary wave is of particular interest in the case of flies, because there exist two rhabdomere types, six wider peripheral rhabdomeres and two more slender central rhabdomeres. As a consequence of this difference in radius the two types of rhabdomere differ also as to their waveguide properties, a property probably essential to the colour vision of flies (Snyder and Pask, 1973b).

Recently we have investigated the visual pigment contained in the rhabdomeres by estimating in vivo difference spectra (Stavenga *et al.*, 1973). In our experiments we have transmitted the test light along the total length of the rhabdomere, thus utilizing the property of the rhabdomere as a waveguide. This technique implies, however, a necessary correction for the inevitable influence of the boundary wave on the measured spectra. After executing this correction with the aid of the refractive index values provided by Seitz (1968) we have been left with discrepancies between the corrected difference spectra of the two rhabdomere types.

There is no reason to doubt Seitz' experimental values, but, as we will discuss in the present paper, waveguide theory leads us to a modified interpretation of the data. The boundary wave also must have interfered in Seitz' estimate of the refractive index of fly rhabdomeres. This effect is treated first. Subsequently a correction method for the boundary wave effect is presented and a more reliable value for the refractive index of the fly rhabdomere is calculated.

B. Rhabdomere Refractive Index Correction Method

With an interference method Seitz (1968) has determined the refractive indices in the fly *Calliphora erythrocephala* (mutants white or chalky). Monochromatic light, transmitted through the medium to be investigated is brought into interference with light having passed a standard medium. The difference in optical path length between the two media has been measured. From the thickness of the media and the refractive index of the standard medium the unknown refractive index can be calculated. The proper way to study the tiny rhabdomeres is to cut slices perpendicular to the rhabdomere axis, and to apply the test beam parallel to it. The crucial point in the refractive index determination of the rhabdomere as performed by Seitz is that his calculations are based on the implicit assumption that the light wave has been propagated completely within the rhabdomere. However, waveguide optics teach us that this assumption is incorrect.

As is derived in the appendix, the effective refractive index n_f of an optic fibre is determined by both the medium in the core and the surrounding medium. If the refractive indices of the media are respectively n_1 and n_2:

$$n_f = K n_1 + (1 - K) n_2. \tag{1}$$

In the appendix the factor K is introduced as

$$K = 1 - U^2/V^2 \tag{2}$$

where U is a function of the fundamental parameter V in waveguide optics, defined as

$$V = 2\pi \frac{\varrho}{\lambda} (n_1^2 - n_2^2)^{\frac{1}{2}} \tag{3}$$

λ is the wavelength of the light in vacuum and ϱ the radius of the waveguide.

$U(V)$ is presented[1] in Fig. 1a as well as $K(V)$, the latter being calculated from (2). The implicit dependence of K on the desired refractive

1 Dr. W. Wijngaard, University of Utrecht, kindly supplied the $U(V)$ as well as the $\eta(V)$-values, which he has calculated to the fourth decimal (in the limit $1 - n_2^2/n_1^2 \to 0$); compare Biernson and Kinsley (1965).

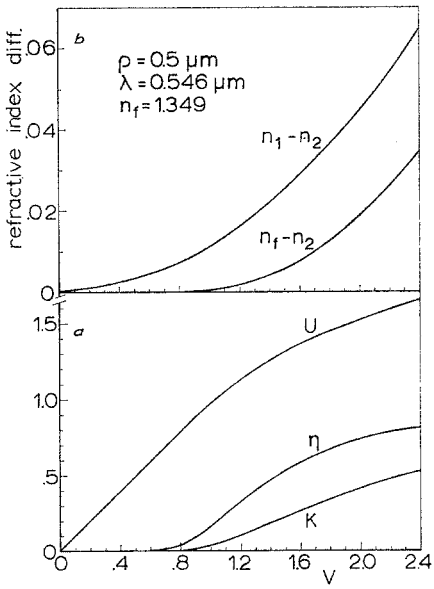

Fig. 1a and b. Waveguide parameters plotted vs the dimensionless frequency $V = 2\pi\varrho\,[n_1^2 - n_2^2]^{\frac{1}{2}}/\lambda$. λ is the wavelength, ϱ the radius of the waveguide and n_1 respectively n_2 the refractive indices of waveguide and surrounding medium. a) U is the argument, at the boundary, of the Bessel-function, describing the electromagnetic field in the waveguide. η is the fraction of light power within the fibre and $K = 1 - U^2/V^2$ the correction factor derived in the appendix. b) Refractive index differences in a waveguide of radius $\varrho = 0.5$ μm. n_f is the effective refractive index of the fibre, see (1)

index n_1 hampers a straightforward solution of n_1 from (1). A direct way to overcome this difficulty is the following method. We rewrite (3) as

$$n_1 - n_2 = \frac{1}{n_1 + n_2}\left[\frac{\lambda V}{2\pi\varrho}\right]^2 \tag{4}$$

$n_1 + n_2$ can be regarded as approximately constant. Then $n_1 - n_2$ is a quadratic function of V (see Fig. 1b). Since relation (1) is equivalent to $n_f - n_2 = K(n_1 - n_2)$ multiplication of $n_1 - n_2$ by K yields the functional dependence of $n_f - n_2$ on V. Thus, knowing $n_f - n_2$ we can immediately derive the corresponding $n_1 - n_2$ (and V) from the graph.

We illustrate here the method for the case of ultimate interest since Fig. 1b is calculated with the experimental data of Seitz (1968), $\lambda = 0.546$ μm and $\varrho = 0.5$ μm. We assign to $n_1 + n_2$ the value $n_1 + n_2 = 2.7$. The value determined for the rhabdomere refractive index has been $n_f = 1.3490(7)$.

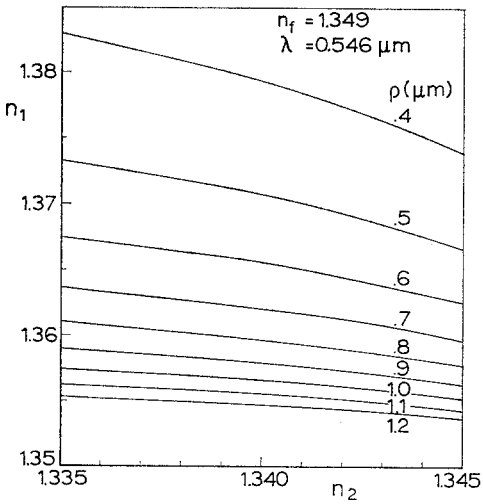

Fig. 2. Relation between the refractive indices of the medium within the waveguide and its surroundings n_1 and n_2 respectively in waveguides of different radii ϱ, which have an effective refractive index $n_f = 1.349$

Now we have to discuss first the value to be taken for n_2. The fact is that a fly rhabdomere is not surrounded by a homogeneous substance but by cytoplasm in the visual cell and by extracellular fluid on its outside. The refractive index of the photoreceptor cytoplasm is 1.3410(8) distally, 1.3400(6) in the neighbourhood of the nucleus and 1.3417(9) more proximally. The extracellular fluid between the rhabdomeres has a refractive index of 1.3365 (Seitz, 1968).

For the present we take $n_2 = 1.339$ or $n_f - n_2 = 0.010$. From Fig. 1b we then obtain $n_1 - n_2 = 0.032$ or $n_1 = 1.371$. This means a striking increase of the refractive index value of the rhabdomere compared with the former value of 1.349.

Taking this corrected value as a starting point, we can subsequently determine how sensitive it is to the choice of the parameter values.

Firstly, the value of the rhabdomere radius given by Seitz as 0.5 μm, probably is on the low side. According to Boschek (1971) in the fly *Musca domestica* the central rhabdomeres have a constant radius well over 0.5 μm while the peripheral rhabdomeres taper from distal to proximal, the radius decreasing from about 1.0 to 0.5 μm.

Secondly, as we have stated, the refractive index of the surroundings of the rhabdomere lies somewhere between 1.336 and 1.342.

With the procedure outlined above we can directly obtain the dependence of the corrected n_1 value on a variable n_2 value, in rhab-

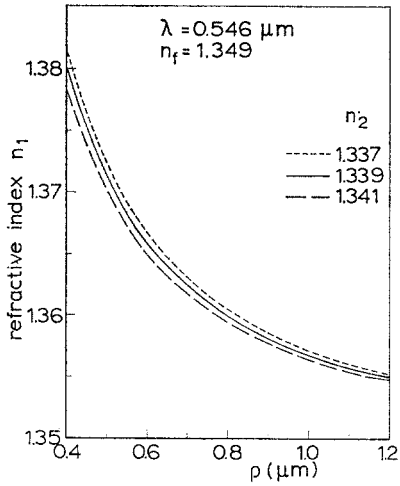

Fig. 3. Refractive index n_1 of the medium within an optical waveguide as a function of radius ϱ when the effective refractive index $n_f = 1.349$ for the cases where the refractive index n_2 of the boundary medium is respectively 1.337, 1.339 and 1.341

domeres with different radii (with $\lambda = 0.546$ μm and $n_f = 1.349$). As shown in Fig. 2 n_1 depends to a small extent on n_2 at least in the case of a wider rhabdomere. On the other hand a rather strong dependence on the radius ϱ becomes manifest. The relation between the corrected refractive index n_1 and the radius of the rhabdomere is visualized in Fig. 3. Three cases are drawn with n_2 equal to 1.337, 1.339, and 1.341 respectively. From this graph it is quite obvious that the effect of n_2 on the value of n_1 is almost negligible compared with that of the radius. Hence, let us confine ourselves to the radius and assume that the radii of the rhabdomeres, in which Seitz has determined a refractive index $n_f = 1.349$, have ranged between 0.5 and 0.8 μm. Then we conclude that an appropriate estimate of the corrected refractive index in fly rhabdomeres is $n_1 = 1.365 \pm 0.006$.

C. Discussion

A fly rhabdomere acts as a waveguide as a consequence of its refractive index being higher than that of its surroundings. We have shown that the experimentally determined refractive index has been affected by the very fact of waveguide behaviour. With a simple method in which is corrected for waveguide effects the real refractive index value can be determined from the experimental value. We next review the presuppositions used in our correction method.

I. The Feasibility of the Correction Method

The factor K, as presented in Fig. 1a, is valid for circle-cylindrical waveguides. The peripheral rhabdomeres of the housefly taper and the cross-section is not a circle (Boschek, 1971). However, if the tapering angle is slight, a local approximation with a cylinder is allowed when the refractive index difference between the rhabdomere and the surrounding medium is small (Snyder and Pask, 1973a). The rhabdomere segments in the 10 μm sections used by Seitz (1968) in his measurements therefore can be treated as uniform. Furthermore, non-circular dielectric waveguide studies (Kapany and Burke, 1972) have revealed that the light propagation of the dominant mode is similar to that in circle-cylindrical fibres. So the more or less elliptical cross-sections of the rhabdomeres are permissibly approximated by a circle. (We note here, that in Seitz' experiments always only one mode, the so-called HE_{11}-mode, will have been present.)

We thus conclude that the deduced value of $n_1 = 1.365 \pm 0.006$ indeed is reliable. The existing discrepancy between the value 1.349 reported by Seitz (1968) with the range 1.37–1.38 calculated by Kirschfeld and Franceschini (1969) on the basis of membrane densities thus is largely solved.

The inaccuracy in the corrected value mainly is the consequence of the inaccuracy in the right value of the radii. The uncertainty concerning the value to be taken for n_2, the refractive index of the medium surrounding the rhabdomere is shown to be of minor importance.

All the same n_2 deserves attention since this parameter influences the waveguide properties with equal weight as n_1 does; see formula (3). We shall consider this aspect more closely.

II. The Media Surrounding the Rhabdomere

A fly rhabdomere is bordered on one side by extracellular fluid with refractive index 1.3365. On the opposite side the photoreceptor cytoplasm has a refractive index of about 1.341, which only holds for the light-adapted state according to Seitz (1970). He has described vesicles arising during dark adaptation in the cytoplasm and assumes that these vesicles result in a refractive index 1.3385 in the cell near the rhabdomere. Seitz argues subsequently that the refractive index variation results in a change in critical angle and thus in a change in transmitted light flux of about 30% (or 0.1 log unit). The vesicles system therefore might be a light control or pupil mechanism.

Seitz (1970) has based his analysis on geometrical optics. We have considered the influence of the refractive index change on the pertinent waveguide optical parameters.

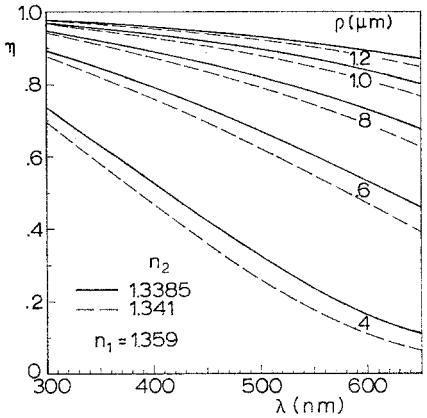

Fig. 4. Wavelength dependence of the light power fraction η within the boundary of the waveguide at varying values of the radius

Firstly, the numerical aperture of an optic fibre is given by $(n_1^2 - n_2^2)^{\frac{1}{2}}$ (Kapany, 1967). Let us adopt the corrected n_1 and use its lowermost value $n_1 = 1.359$. Then the relative variation is extreme, but still a change in n_2 from 1.341 to 1.3385 only enlarges the numerical aperture with a factor 1.07.

Secondly, a most important parameter in the optics of visual waveguides is the fraction η of light power propagated within the core. The relation of η to V already is presented in Fig. 1a. It is an easy task to compute from $\eta(V)$ the dependence of η on wavelength in the case of a fly rhabdomere. Fig. 4 shows $\eta(\lambda)$ for rhabdomeres with radii 0.4 to 1.2 μm and $n_1 = 1.359$. Each case is calculated for respectively $n_2 = 1.341$ and $n_2 = 1.3385$. Clearly the fraction η of light intensity within the rhabdomere is only slightly different for the two refractive index values at all but the smallest radii.

So, we conclude that from the optics it is difficult to estimate the light control function of the vesicles system. Moreover, Seitz has detected the vesicles after glutaraldehyde prefixation followed by freeze etching, while Boschek (1971) notes that no such entities have been observed using classical fixation techniques.

Nevertheless, there is an adaptation system which certainly acts as an effective pupil. We refer to the migrating pigment granula system localized in the retinula cells of the fly (wild type). Kirschfeld and Franceschini (1969) first described this pupil mechanism. On illumination, small pigment granula which are dispersed throughout the visual cell during dark adaptation move towards the rhabdomere. The granula

assembled in the immediate neighbourhood of the rhabdomere constitute a (longitudinal) pupil (similar as previously proposed for the superposition eye by Kuiper, 1962).

Again it is the functioning of the rhabdomere as a waveguide that is of principal interest for this phenomenon. While for the generation of the visual signal only the light within the rhabdomere is useful, the pupil mechanism on the contrary must exploit the light propagated outside, i.e. the pigment granula near to the rhabdomere interact with the boundary wave. From this wave light-energy can be absorbed and scattered and so the transmitted light flux is controlled.

Transmission variations of up to 2.5 log units can be measured from the complete length of photoreceptors of the blowfly *Calliphora* (Stavenga, 1971). The effective light-intensity decrease equals about 1.3 log units as follows from calculations on the interaction of the pupil with the visual pigment chemistry (Stavenga *et al.*, 1973).

The clustering of sense cell pigment granula in principle also can affect the refractive index near the rhabdomere. If the light-induced refractive index change indeed is not negligible we must expect that (in addition to the influence on the light fraction η) also the acceptance angle of the visual cell is changed. Streck (1972) has studied the acceptance angle with intracellular recordings of blowfly retinula cells, but has not been able to demonstrate a well-founded dependence on the adaptation state. Vowles (1966) on the contrary has reported long term variations in the visual cell acceptance angle and has related these changes to—not very convincingly—observed adaptation processes in the primary pigment cells. While the reported phenomena have yet to be confirmed by others, it moreover remains obscure in which way primary pigment cell granula can affect the sense cell acceptance angle. Anyhow, the processes described by Vowles are much slower than those inside the visual cells which we have discussed.

After all, it seems to be improbable that the refractive index of the medium surrounding the fly rhabdomere depends strongly on adaptation processes. In view of the presented evidence we may take as an acceptable estimate for the refractive index of the medium surrounding the fly rhabdomeres $n_2 = 1.339 \pm 0.002$.

D. Conclusion

By applying the theory of optical waveguides to the fly rhabdomere we have obtained a corrected value $n_1 = 1.365 \pm 0.006$ for the refractive index of the medium within the rhabdomere. We thus are able to calculate reliably the influence of the waveguide optics on our visual pigment measurements. The results we shall present elsewhere. Since the corrected n_1 value deviates rather much from the formerly reported

one of 1.349, it is to be expected that it will have rather serious consequences for studies based on the old value (for instance, Snyder and Pask, 1973b).

The deduced value $n_1 = 1.365(6)$ probably also holds for other rhabdomeric photoreceptors (Stavenga, 1974; accepted for publication in Vision Res.).

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Appendix

Derivation of the Correction Factor

Consider a waveguide with radius ϱ and refractive indices n_1 and n_2 of waveguide and surrounding medium. It should be recalled that, when an electromagnetic wave, having vacuum wavelength λ is propagated in a medium with propagation constant k, then the refractive index n of the medium is defined as $n = k\lambda/2\pi$.

Now, waves propagating in a dielectric waveguide can be expressed as a sum of a finite number of waveguide modes where each mode has a distinct propagation constant. For a single mode Snitzer (1961) has derived expressions for the electromagnetic field components in the axial direction. The amplitudes in the waveguide are described by a Bessel function of the first kind and in the surrounding medium by a modified Hankel function. The arguments of these functions on the waveguide boundary, being respectively U and W, are related to the propagation constant of the mode along the fibre, k_f, by (Biernson and Kinsley, 1965):

$$[U/\varrho]^2 = k_1^2 - k_f^2 \tag{1}$$

and

$$[W/\varrho]^2 = k_f^2 - k_2^2 \tag{2}$$

where k_1 and k_2 respectively are the propagation constants of the media within and surrounding the waveguide. The dimensionless parameter V is introduced as

$$V^2 = U^2 + W^2 \tag{3}$$

So

$$[V/\varrho]^2 = k_1^2 - k_2^2 \tag{4}$$

(this relation is equivalent to Eq. (3) of Sect. B). We derive from (2) and (4) that

$$\frac{k_f - k_2}{k_1 - k_2} = \left(\frac{W}{V}\right)^2 \cdot \frac{k_1 + k_2}{k_f + k_2} \tag{5}$$

Since $k_f = 2\pi n_f/\lambda$, $k_1 = 2\pi n_1/\lambda$, $k_2 = 2\pi n_2/\lambda$ and $k_1 + k_2 \simeq k_f + k_2$ we obtain

$$n_f - n_2 = K(n_1 - n_2) \tag{6}$$

where

$$K = \left(\frac{W}{V}\right)^2 = 1 - \left(\frac{U}{V}\right)^2 \tag{7}$$

(6) can also be written as

$$n_f = Kn_1 + (1 - K)n_2 \tag{8}$$

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